EXTRACTION OF ASCORBIC ACID FROM FRESH PINEAPPLE

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"I declare that this thesis entitled "*Extraction of Ascorbic Acid From Fresh Pineapple*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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To my beloved mother, father, younger brother, sisters and Jasmafazli

Thank you for your supporting.

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ABSTRACT

Pineapple fruits have a high concentration of ascorbic acid, nowadays everybody know it as Vitamin C in their composition. Today, ascorbic acid is one important supplement to take care of health. . There are a lot of benefits that can get from ascorbic acid. Before the extraction of ascorbic acid from fresh pineapple, the fresh pineapple was cuted and cleaned. Pineapple needed to blend and the filtered. After that, the sample is being mix with the solvent extraction, which is .0.02 M potassium phosphate, pH 2.5 by phosphoric acid : acetonitrile (98 : 2.). The mixers then sonicated in ultrasonic extraction follow the time that are determined. There are two objectives in this research, first to determine the optimum extraction time. The parameters that will use are 10 minutes, 20 minutes and 30 minutes. Then the other one is to determine the optimum amount of sample. The parameters that used is 20 ml, 40 ml, and 60 ml of sample. Then the samples are filtered again before analysis. High Performance Liquid Chromatography (HPLC) was used to analyze where there are contain or not ascorbic acid in fresh pineapple. The HPLC method was used to developed and validated for determining the amount of ascorbic acid in fresh pineapple. The mobile phase that used in HPLC is 0.02 M potassium phosphate, pH 2.5 by phosphoric acid and acetonitrile. Ascorbic acid was determined at range one to two minutes and gets two peaks. The first peak is l ascorbic acid and the second one is dehydroascorbic acid (DHA). This research finally obtained the objective of the research.

ABSTRAK

Buah nanas mempunyai kandungan ascorbic acid yang tinggi. Pada masa sekarang semua golongan mengetahui bahawa ascorbic acid terdapat dalam buah nanas. Pada hari ini, ascorbic acid merupakan vitamin yang terpenting sekali kepada tubuh badan manusia. Sebelum pengekstrakan dilakukan, buah nanas yang segar hendaklah dibersihkan dan dipotong keci. Selepas itu, buah nanas tadi hendaklah dikisar halus dan ditapis untuk mendapatkan jusnya. Kemudian, hasil tapisan tadi dicampur dengan 0.02 M photassium phosphate, pH 2.5 dari phosphoric acid : acetonitrile (98 :2). Campuran itu kemudiannya dimasukkan kedalam " ultrasonic extraction " untuk diuji dengan masa yang berbeza. Dalam kajian ini, terdapat dua parameter yang hendak diuji yang mana pertamanya adalah untuk menguji masa yang terbaik untuk pengektrakan iaitu dengan masa 10,20 dan 30 minutes. Keduanya, adalah untuk mengetahui kandungan terbaik sample yang mengandungi ascorbic acid dalam 20,40 dan 60 ml sampel. Sampel sampel ini kemudiannya ditapis sekali lagi sebelum dianalisis dengan menggunakan " High Performance Liquid Chromatograph(HPLC) " yang mana digunakan untuk menguji kehadiran ascorbic acid di dalam sampel buah nanas. Sekiranya terdapat kehadiran ascorbic acid di dalam sampel, graf akan terhasil. Bahan pengantara yang digunakan dalam HPLC ini adalah acetonitrile dan 0.02M potassium phosphate, pH 2.5 dari phosphorik acid . Hasil analisi telah membuktikan kewujudan ascorbic acid dalam buah nanas pada masa satu hingga dua minit dan menghasilkan dua graf. Graf yang pertama ialah "l ascorbic acid" dan yang kedua ialah "dehydroascorbic acid" (DHA).

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%	Percent
µl	micro liter
g	Gram
g/ml	gram per millimeter
kg	Kilogram
mAu	milli Absorbance
ml	Mililiter
Mm	Millimeter
º C	Celcius
ppm	part per million
v/v	volume per volume

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CHAPTER 1

INTRODUCTION

1.0 Introduction

Pineapple (Ananas comosus L. Merrill) fruit cropping and processing are important horticultural industries in many countries with tropical climates. In terms of worldwide production, pineapple is currently the third most important tropical fruit after bananas and mangoes. Pineapple is cultivated mainly for fresh or canned fruit and juice, but also the only source of bromelain, a complex proteolytic enzyme used in the pharmaceutical market and as a meat-tenderizing agent [1].

As individual fruits develop from the flowers, they join together forming a cone shaped, compound, juicy, fleshy multiple fruit of approximately 30cm or more in height. Pineapple maturity is evaluated by the fruit eye flatness, the extent of skin yellowing and by aroma. It takes 3-5 months for pineapple fruit to reach maturity and pineapple fruit quality is highest if the fruit matures on the plant. Pineapples harvested prematurely do not continue too ripen or sweeten, as there are no starch reserves in the fruit to be converted to sugar.

A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. Numerous studies were carried out on some of these plants, e.g. rosemary, sage, oregano, which resulted in a

development of natural antioxidant formulation for food, cosmetic and other applications [1]. However, scientific information on antioxidant properties of various plants, particularly those that are less widely used in culinary and medicine is still rather scarce. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and neutraceuticals.

Antioxidants play an important role in manufacturing, packaging and storing fats and fatty foods. Numerous compounds, both synthetic and natural in origin, have been developed as antioxidants for food preservation, but only tert-butyl-4-hydroxyanisol(BHA) and tert-butyl-4-hydroxytoluene(BHT) and tocopherol is natural antioxidants are practically used [3]. However, the most widely used antioxidants, BHA and BHT, are suspected of causing liver damage. On the other hand, tocopherols are widely used as safe natural antioxidants, but they are not as effective as synthetic antioxidants and the manufacturing cost is high. These circumstances stimulated the isolation of an antioxidant from natural sources, especially from plant materials [3].

Pineapple contains a lot of ascorbic acid which is a water-soluble vitamin that has a number of biological functions. Acting as an antioxidant, ascorbic acid important functions is to protect Low-density Lipoprotein (LDL) cholesterol from oxidative damage. Only when LDL is damaged does cholesterol appear to lead to heart disease, and ascorbic acid may be one of the most important antioxidant protectors of LDL. Ascorbic acid may also protect against heart disease by reducing the stiffness of arteries and the tendency of platelets to clump together [2,5].

Ascorbic acid is needed to make collagen, the “glue” that strengthens many parts of the body, such as muscles and blood vessels. Ascorbic acid also plays important roles in wound healing and as a natural antihistamine. This ascorbic acid

also aids in the formation of liver bile and helps to fight viruses and to detoxify alcohol and other.

Ascorbic acid is an antioxidant that is required for tissue growth and repair, adrenal gland function, and healthy gums. It enhances immunity, protects against bruising and promotes the healing of wounds, also aid in interferon production. Ascorbic acid works well with vitamin E [5].

1.1 Objective

The objective of the research is to:

Extract the ascorbic acid from flesh pineapple (Ananas Comusus).

1.2 Scope

To achieve the objective of this research, there are two scopes that have been identified:

i. To determine the optimum amount of sample.
ii. To determine the optimum extraction time.

1.3 Problem Statement

The antioxidant properties of ascorbic acid are thought to protect smoker, as well as people expose to second hand smoke, from the harmful effects of free radicals. Oxidative rancidity is initiated by oxygen free radicals. Oxidation may be prevented or delayed by antioxidants. Synthetic antioxidants are approved as food additives, international regulations tend to establish more and more restrictions so their use and the consumer increasingly prefers to avoid synthetic additives in flavor of those perceived of natural. Concerning about it safety to human body is encouraging research on substances from natural origin showing antioxidant properties.

There has been an interest by the industry and a desire by consumers to replace synthetic compounds with natural antioxidant alternatives. It can reduce the cost of industry, making antioxidant. We can use the natural alternatives sources of antioxidant for our health. We also can identification of sources by using laboratory equipment such as HPLC and also GC. Lastly, the extraction of ascorbic acid from pineapple can expands the usage of pineapple and it can reduce the cost in production of antioxidant in Malaysia. Malaysia can produce their own ascorbic acid rather than made it from grape seed fruits and orange fruits.

1.4 Research Planning

In this research, there is a lot of work to be done to make this research complete. The first chapter contains the introduction, objective of the research, scope to complete, and problem statement. In second chapter, they include all the literature review of the research such as what is antioxidant, free radical, history of pineapple, ascorbic acid, separation, and extraction and also the analysis research.

All the information about the method to run in this research contains in chapter three. They are such as how to prepare raw material, how to extract, how to make standard curve of ascorbic acid and also how to analyze the sample. Then, chapter four is result and discussion. It contains all result that had been obtained from the analysis done. This chapter also explain about the graph obtain and also the problem occurs during handling this research. Lastly, in chapter five are the conclusion and recommendation about this research for better study next time.

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

In this chapter, the entire previous journal will be seeing to find information about the research. Firstly, it is difficult to find the journal that extracts the ascorbic acid from pineapple. Mostly, orange, grape seed fruits and guava is used. Therefore, these researchers need to improve that in pineapple also contains ascorbic acid.

2.1 Antioxidant

Antioxidants are the family of chemical compounds that protects cells in the human body against oxidation damage, caused by oxygen free radicals that have become electrically charged. The damage these oxygen free radicals cause has been linked to many illnesses, including aging, heart disease, cancer, and two diabetes. In the world of nutritional supplements, antioxidant is becoming synonymous with anti-aging. ORAC, or Oxygen Radical Absorbance Capacity, is a new standard of measuring the ability of a compound to prevent oxygen free radical damage [6].

Antioxidant is a substance that prevents or slows the breakdown of another substance by oxygen. Synthetic and natural antioxidants are used to slow the

deterioration of gasoline and rubber, and such antioxidants as vitamin C (ascorbic acid), butylated hydroxytolune (BHT), and butylated hydroxyanisole (BHA) are added to foods to prevent them from becoming rancid or form discoloring [7].

In the body, nutrients such as beta-carotene, a vitamin A precursor, vitamin C. vitamin E, and selenium have been found to act as antioxidants. They act by scavenging free radicals, molecules with one or more unpaired electrons, which rapidly react with other molecules, starting chain reaction in a process called oxidation. Free radicals are a normal product of metabolism; the body produces its own antioxidants for example the enzyme superoxide dismutase to keep them in balance [8].

However, stress, aging, and environmental sources such as polluted air and cigarette smoke can add the number of free radicals in the body, creating an imbalance. The highly reactive free radicals can damage healthy DNA and have been linked to changes that accompany aging such as age-related macular degeneration, a leading cause of blindness in older people and with disease processes that lead to cancer, heart disease, and stroke.

Many antioxidants, including vitamin C and vitamin E cannot get into mitochondria for various reasons, for example it is because they are too hydrophilic to cross mitochondrial membranes or too hydrophobic to cross the cytoplasm. A group of scientists in Russia led by V. Skulachev have created a custom antioxidant a Skulachev ion forms the point of the molecule and penetrates the mitochondrial membranes; the antioxidising part is attached behind it and that can the mitochondria and stays there due to the membrane potential gradient; preventing damage to DNA [9].

Although there is little doubt that antioxidants are necessary component for good health, there is considerable doubt as to the most beneficial antioxidant and as

to the optimal amount for the results. A study of lung cancer patients found that those given antioxidant supplements had worse prognoses This is believed to be due to antioxidant interference with the body's normal use of localized free radicals for example nitric oxide for cell signaling. Due to the complex nature of the interactions of antioxidants with the body, it is difficult to interpret the results of many experiments designed to test such things. In vitro testing outside the body has shown many natural antioxidants, in specific concentration, can halt the growth of or even kill cancerous cells [9,10]

Antioxidants compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sources food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidants compounds in a typival diet are derived from plant sources and belong to the variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidants activiry, while others, such as the mono-phenols are weak antioxidants [11].

2.2 Free Radical

The definition of free radical in chemistry is a molecule or atom that contains an unpaired electron but is neither positively nor negatively charged. Free radicals are usually highly reactive and unstable. They are produced by homolytic cleavage of a covalent bond. Each of the atoms connected by the bond retains one of the two electrons making up the bond. The homolytic cleavage of a hydrogen molecule, H_2, produces two hydrogen free radicals hydrogen atoms. Similarly, two chlorine free radicals can be produced from a chlorine molecule. Homolytic cleavage of the

carbon-bromine bond in methyl bromide, CH_3Br, would produce a methyl free radical and a bromine free radical. The term free is often dropped in referring to the *free* radicals; this could lead to conclusion if the term *radical* were used synonymously with *group* in organic chemistry, by calling an alkyl group an alkyl radical when free radical was not intended [7].

Free radical production is actually a normal part of life, part of the equation of simply breathing in oxygen. Usually, the body's natural defense systems neutralize free radicals that develop, rendering them harmless. However, environmental assaults on the body, such as UV-radiation, pollutants and alcohol, can overpower the body's ability to neutralize free radicals, allowing them to cause damage to the structure and function of the body's cells [13]. There is good evidence that this damage contributes to aging and leads to a host of illness, including cancer and heart disease.

The free-radical theory of aging is that organisms age because cells accumulate free radical damage with the passage of time. For most biological structures free radical damage is closely associated with oxidation damage. Oxidation and reduction are redox chemical reactions. Most people can equate to oxidation damage as they are familiar with the process of rust formation of iron exposed to oxygen. Oxidation does not necessarily involve oxygen, after which it was named, but is most easily described as the loss of electrons from the atoms and molecules forming such biological structures. The inverse reaction, reduction, occurs when a molecule gains electrons [9,14] .

In biochemistry, the free radicals of interest are often referred to as "reactive oxygen species" (ROS) because the most biologically significant free radicals are oxygen-centered; however it should be noted that not all free radicals are ROS and not all ROS are free radicals [7].

2.3 Natural Antioxidant

A natural antioxidants protects the body against free radicals. A natural antioxidant is an anti-oxidizing substance that protects the body from oxidation, a process that can cause serious damage to cells. Natural antioxidants can be derived from plants [7].

Groups of well-known natural antioxidants include catechins, coumarins, indoles, and caretenoids. Carotenoids are the largest group of naturally occurring antioxidants [9]. Carotenoids is a plant pigments with vitamin-like properties that give color to brightly colored vegetables, like squash, carrots, peppers and tomatoes [14].

Beta-carotene is the most common natural antioxidant in the caretenoid group. Beta-carotene supports the immune system, might reduce the skin's risk to sun damage and DNA damage, supports healthy cholesterols levels and increases lung capacity. Some studies show this natural antioxidant supports a healthy heart [14,17].

Natural antioxidant supplements that contain beta-carotene should be taken with care. High doses of beta-carotene over periods of time have produced yellow or orange coloring on hands and feet. This natural antioxidation may also cause nausea, loose stools, and bruising and joint pain [16].

Vitamins C and E, both a natural antioxidant, can help to limit beta-carotene's oxidation or help it recycle so that when it's oxidized it will not harm cells [7].Other common carotenoids are lutein and lycopene. The body needs lutein to support vision and maintain a healthy heart. The lutein found in natural antioxidants supplements is extracted from marigold flower petals. Lycopene, another natural antioxidant, protects the heart by lowering LDL cholesterol [15,18].

2.3.1 Classification of Major antioxidants

There are many type of antioxidant such as enzymes or natural antioxidant from vitamins. The table 2.1 below shows that the classification of major antioxidant.

Table 2.1 : The classification of major antioxidant.

Enzymes	Antioxidant	Role	Remarks
	Superoxide dismutase (SOD) Mitochondrial Cytoplasmic Extracellular	Dismutases O_2 to H_2O_2	Contains Manganese (Mn. SOD) Contains Copper & Zinc (CuZnSOD)
	Catalase	Dismutases H_2O_2 to H_2O	Tetrametric hemoprotein present in peroxisomes
	Glutathione peroxidase (GSH.Px)	Removes H_2O_2 and lipid peroxides	Selenoproteins (contains Se^{2+}) Primarily in the cytosol also mitochondria Uses GSH
Vitamins	Alpha tocopherol	Breaks lipid peroxidation Lipid peroxide and O_2 and OH scavanger	Fat soluble vitamin
	Beta carotene	Scavanger OH,O_2 and peroxy radicals	Fat soluble vitamin

		Prevents oxidation of vitamin A Binds to transition metals	
	Ascorbic acid	Directly scavenges O_2,OH, and H_2O_2 Neutralizes oxidants from stimulated neutrophils Contributes to regeneration of vitamin E	Water soluble vitamin

Modified from Fisher, 1988. Submitted by Dr. Tamer Fouad, M.D [19]

2.3.2 Nutritional Antioxidants

The following substances have shown positive antioxidants effects [8] on the body. Nutritional antioxidant brings more benefits to the human body. There are listed type of nutritional antioxidant below.

i. Vitamin A or *Retinol*, also synthesized by the body from beta-carotene protects dark green, yellow and orange vegetable and fruits from solar radiation damage. It is though to play a similar role in the human body. Carrots, squash, broccoli, sweet potatoes, tomatoes, kale, collards, cantaloupe, peaches and apricots are particularly rich sources of beta-carotene.

ii. Vitamin C or Ascorbic acid is a water soluble compound that fulfils several roles in living system. It is important sources include citrus fruits such as oranges, sweet lime, all yellow fruits, potatoes, pineapple, green peppers, broccoli, green leafy vegetables, strawberries, raw cabbage, and tomatoes.

iii. Vitamin E including *Tocotrienol* and *Tocopherol* is a fat soluble and protects lipids. Sources include wheat germ, nuts, seeds, whole grains, green leafy vegetables, vegetable oil, and fish liver oil.

iv. Selenium has been shown as to have beneficial effects in reducing the occurrence of male prostate cancer. However, the substances must be taken in measured amounts because large doses of the elements can be toxic. Good food sources include fish, shellfish, red meat, grains, eggs, sunflower seeds, chicken, garlic, and Brazil nuts. Vegetables can also be a good source if they are grown in selenium-rich soils and some nutritional supplements contain a supply of selenium.

v. Bio-flavonoids are present in many dark berries such as pomegranate, noni, blueberries and blackberries as well as in certain types of tea and coffee especially green tea.

2.4 Pineapple

2.4.1 The History of Pineapple

The pineapple, *Ananas comosus* Merr. is a member of the Bromeliaceae, a large, diverse family of about 2000 species. Bromeliads, with the exception of one species (*Pitcairnia feliciana*), are native only to the new world, largely in the tropics showed in figure 2.1. Most of the 50 or so genera are composed of epiphytic species, the exception being Ananas (pineapple) and a few others such as Bromelia and Pitcairnia. Family members are further distinguished by being herbaceous and rosette-forming, with stellate hairs on appendages of and colored floral bracts. The family contains hundreds of taxa used in as ornamentals in greenhouse or sub-tropical areas : *Billbergia*, *Vresia*, *Nidularium*, *Aechmea*, *Guzmaria*, *Pitcairnia*, and *Tillandsia*. *Tilladsia usneoides* is Spanish moss native to the Gulf States. The related species *A. ananassoides* and *A. bracteus* have been used to a limited extent in pineapple breeding. Formerly, the pineapple was named *Ananas sativus*, *Bromelia ananas*, or *Bromelia comosus*. Like the banana, it is one of the few important fruitimg monocots. [20,21].

Figure 2.1: Pineapple

The pineapple is native to southern Brazil and Paraguay where wild elatives occur. And it was spread by the Indians up through South and Central America to the West Indies before Columbus arrived. In 1943 Columbus found the fruit on the island of Guadaloupe and carried it back to Spain and it was spread around the world on sailing ships that carried it for protection against scurvy. The Spanish introduced it into the Philipines and may have taken it to Hawaii and Guam early in the 16th century. The pineapple reached England in 1660 and began to grown in green houses for its fruits around 1720[22,2,24].

In the natural form, every variety of pineapple has rough, diamond-pattern skin. Their tasted vary slightly; through they all basically have the same juicy, tart taste. Pineapple is grown al year long in the warmer climates. The pineapple plant is an herbaceous perennial that grows to be two to five feet high, and three to four feet across. It has a short, thick stem with waxy leaves[25,26].

Pineapples are usually grown by propagation. That is, they are grown by replanting a part of themselves. The four common parts are; the slips which is located on the stem below the fruits, the suckers that start at the leaves, the crowns the leafy growth on top of the pineapple, and the ratoons that are located on the roots[26].

The pineapple is native to dry forest or thorn scrub vegetation regions of South America; although it is exact origin is disputed. Older sources placed the center of diversity in southern Brazil and Paraguay, but more recent study suggests it may be northern Brazil, Columbia, and Venezuela. In part the confusion stems from distribution of cultivated types by Indians probably distributed it to Guadeloupe, where it was collected by Columbus in 1943. The pineapple was then taken to Europe and distributed to the Pacific islands, India and Africa by Spaniards and the Portuguese explorers of the 16^{th} and 17^{th} centuries.

The first commercial plantation was established on Oahu in 1885 and Hawaii produced most of the world's pineapple until 1960's, when urbanization and society of labor forced production elsewhere, particularly the Philippines. The Hawaiian industry has continued its slow decline over the last decade and now produces only 2% of the world's pineapple. Florida produced pineapple for a short time period at the turn of the 20^{th} century but freezes in 1917 devastated the industry. Pineapple was first canned in 1888 in Malaysia and canned fruits were first exported from Singapore in about 1900. Today, Southeast Asia still dominates the world production but large amount are produced in Latin America and Africa as well.

2.4.2 Composition in Pineapple

Every fruits have their own compositions and their nutrient such as vitamin C, zink, calcium and many more. Pineapples also have their own nutrients. There are listed below in table 2.2.

Table 2.2: Composition contents in pineapple per 100 grams

Nutrients	
Water (g)	86.50
Calories	49.00
Protein, g	0.39
Fat, g	0.43
CHO: total, g	12.39
CHO: fiber, dietary, g	0.54
Ash, mg	0.29
Calcium, mg	7.00
Phosphorus, mg	7.00
Iron, mg	0.37
Sodium, mg	1.00
Potassium, mg	113.00
Vitamin A, IU	23.00
Thiamine, mg	0.092
Riboflavin, mg	0.036
Niacin, mg	0.42
Ascorbic acid, mg	15.40

(Sourcess:http://food.oregonstate.edu/a pine.html)

2.5 Ascorbic Acid

Vitamins are substances that play an essential part in animal metabolic processes, which the animals can not synthesis. In their absence the animal develops certain deficiency diseases or other abnormal conditions. Vitamins are chemicals other than proteins, carbohydrates, fats and mineral salts that are essential constituents of the food of animals [29].

Ascorbic acid is familiar known as Vitamin C is a water-soluble vitamin that has a number of biological functions. Ascorbic acid is the common name for Synthetic Vitamin C which is used by most vitamin companies. Other forms include; Calcium Ascorbate or Buffered Vitamin C and Sodium Ascorbate or Buffered Vitamin C [30].

Ascorbic acid (2,3-endiol-L-gulonic acid-γ-lactone) also called vitamin C or L-ascorbic acid, is a strongly reducing the dibasic acid with a Pk_1 of 4.1 and pK_2 of 11.8. It is easily oxidized by several agents, especially in aqueous solutions, to form dehydroascorbic acid (DHA) via a semidehydroascorbic acid which can be reduced to ascorbic acid, for instance by sulfur containing agents like glutathione [11].

Ascorbic acid has been reported to reduce activity of the enzyme, aldose reductase, Vincent TE. Aldose reductase is the enzyme responsible for accumulation of sorbitol in eyes, nerves, and kidneys of people with diabetes. This accumulation is believed to be responsible for deterioration of these parts of the body associated with diabetes. Therefore, interference with the activity of aldose reductase theoretically helps protect people with diabetes [31].

Ascorbic acid is found in berries, citrus fruits like strawberry, orange, pineapple and green vegetables. Ascorbic acid is also found in herbs such as alfalfa, burdock root, cayenne, chickweed, eyebright, fennel seed, fenugreek, hops, horsetail, kelp, peppermint, mullein, nettle, oat straw, paprika, parsley, pine needle, plantain, raspberry leaf, red clover, rose hips, skullcap, violet leaves, yarrow and yellow dock [32].

Ascorbic acid may help protect the body against accumulation or retention of the toxic mineral, lead. In one preliminary study, people with higher blood levels of vitamin C had much lower risk of having excessive blood levels of lead [33]

Ascorbic acid is synthesized from vegetable starch which is then converted to glucose by enzymatic treatment. The glucose is then converted to sorbitol by catalytic hydrogenation. The sorbitol is then fermented forming sorbose. Sorbose is then reacted with acetone and sulfuric acid. This is then oxidized with sodium hydroxide and a catalyst [34].

Vitamin C or Ascorbic Acid is the enolic form of 3-oxo-L-gulofuranolactone. It can be prepared by synthesis from glucose or extracted from plant sources such as rose hips, blackcurrants or citrus fruits. It is easily oxidized in air. It is essential for the formation of collagen and intercellular material, bone and teeth and for the healing of wounds. It helps maintain elasticity of the skin aids the absorption or iron and improves resistance to infection. It is used in the treatment or scurvy. It may prevent the occurrence and development of cancer [29]

Ascorbic acid is widely biosynthesized in nature, particularly by chlorophyll containing plants and by animals with the exception of a few mammalian and avian species [12]. A wide variety of a good ascorbic acid's sources has been summarized and listed by Friedrich [11]. Potatoes and citrus fruits are quantitatively is the most important sources of ascorbic acid.

2.5.1 Structure of Ascorbic Acid

The physical properties of ascorbic acid are listed below in table 2.3 and there are comparable for the natural and the synthesis of vitamins. Properties relating to ascorbic acid's structure were recently reviewed by Tolbert and co-workers [35,49].

Table 2.3: The physical properties of Ascorbic Acid

Property	Characteristics
Appearance	White, odorless, crystalline solid with a sharp acidic taste.
Formula	$C_6H_8O_6$
Molecular weight	176.13
Crystal form	Monoclinic; usually plates,sometimes needles
Melting point	190 – 192 ºC (decomposition)
Density	1.65
Optical rotation	$[\alpha]^{25}$ + 20.5º to 21.5º (C = 1 in water) $[\alpha]^{23}$ + 45º (C = 1 in water)
Ph	3 (5 mg/ml) : 2 (50 mg/ml)
pK_1	4.17
pK_2	11.57
Redox potential	First stage: $E_\circ$ + 0.166 V (pH 4)
Solubility	1 g dissolves in 3 ml water, 30 ml 95% ethanol, 50 ml absolute ethanol, 100 ml glycerol USP, or 20 ml propylene glycol. Insoluble in ether, chloroform, benzene, petroleum ether, oils, fats and fat solvents.
Spectral properties Ultraviolet	pH 2 : E_{max} (1 %, 1 cm) 695 at 245 nm (non-dissociated form) pH 6.4 : E_{max} (1 %, 1 cm) 940 at 265 nm (monodissociated form)
Infrared (KBr)	Characteristics wavelength cm^{-1} : 3455, 3405, 3155, v OH groups 2570 associated Oh groups 1770, 1670 carbonyl lactones 1254 C-O-C lactones 1057 δ OH groups
Structure of ascorbic acid	

2.5.2 Sources of Ascorbic Acid

Ascorbic acid occurs in significant amounts in vegetables, fruits and animal organs such as liver, kidney and brain. Potatoes and cabbage are probably the most important sources of ascorbic acid for the majority of the Western population. Today, fresh fruits and vegetables are available all year. During storage and cooking, part of the ascorbic acid is lost due to oxidation. Only trace amounts of ascorbic acid are contained in milk, grains and meat found. Ascorbate values of up to 300 mg/100 g were measured in several tropical fruits. Neotropical plants eaten by primates and herbivorous bats accumulate ascorbic acid up to a concentration of 585 mg/100 g fresh weight with average between 46.5 and 96.3 mg/g depending on the parts (fruits, foliages or flowers), providing the monkeys with about 100 mg/kg body weight/day and bat with 260 mg/kg body weight/day [36,40]. The ascorbic acid contents of some representative foods are listed below in table 2.4

Table 2.4 : The Contents of Ascorbic Acid in Selected Foods

Fruits and Meat	Ascorbic Acid (mg/100 g)	Vegetables	Ascorbic Acid (mg/100 g)
Fruits of *Terminalia fernandiana*	3000	Peppers	125-200
Acerola	1300	Kale	120-180
Rose hips	1000	Parsley	170
Howthorn berries	160-800	Turnip greens	139
Guava	300	Horseradish	120
Black currant	150-230	Collard greens	100-150
Lemons	50-80	Brussels sprouts	90-150
Strawberries	40-90	Broccoli	70-160
Oranges	40-60	Spinach	50-90
Grapefruits	35-45	Watercress	79
Red currant	40	Cauliflower	60-80
Tangerines	30	Kohlrabi	66
Pineapple	20-40	Cabbage	30-60
Rasberries	18-25	Turnips	15-40
Melons	13-33	Asparagus	15-30
Apples	10-30	Leek	15-30
Cherries	10	Potatoes	10-30
Peaches	7-14	Beans	10-30
Bananas	5-10	Peas	10-30
Liver, kidney	10-40	Onion	10-30
Fish	0-3	Tomatoes	10-30
Meat (beef,port)	0-2	Squash	8-25
Meat		Corn (sweet)	12
	3-6	Rhubarb	10
Human	1-2	Celery	7-10

Cow			
		Carrots	5-10
		Oat, rye, wheat	0
		rice	0

2.5.3 Distribution and Nutritional Requirements

Ascorbic acid is an essential nutrient for health maintenance. It exists as a pool of ascorbate, distributed throughout the body with specific tissues having high concentration. For example plasma of normal individuals contains 0.8-1.4 mg per 100 ml. Ascorbic acid must be supplied from outside sources as it is not produced in humans. The first uses of the vitamin were to prevent and treat scurvy. Subsequent uses developed from biochemical studies that delineated its extraantiscorbutic activities related to maintain of good general health [36,35]. Table 2.5 below shows that the recommended of ascorbic acid in dairy dietary.

Table 2.5 : Recommended Vitamin C (Ascorbic Acid) Daily Dietary Allowances [36].

Age Group	Amount (mg)
Males (adults : 19-51+ years)	60
Female Adults:19-51+ years Pregnancy, second half Lactation period	 60 80 100
Children Neonates, premature infants Under 1 years 1-3 years 4-6 years 7-10 years 11-14 years 15-18 years	 100 35 45 45 45 50 60
Others Smokers Diabetic, elderly persons, patients suffering from stress or allergies	 100 Up to 200